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Testicular peritubular cells secrete a protein under androgen control that modulates Sertoli cell functions

(mesenchymal–epithelial cell interactions)

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ABSTRACT **Peritubular cells of the seminiferous tubule synthesize component(s) that stimulate Sertoli cells in culture to increase the production of androgen-binding protein and testicular transferrin. The active peritubular cell component(s) are trypsin-sensitive, heat-sensitive, acid-stable molecule(s) having a molecular weight between 50,000 and 100,000. These specific factors(s) are referred to as P Mod-S to designate protein(s), produced by peritubular cells (P), that modulate the functions of Sertoli cells (S). The degree of stimulation by P Mod-S is comparable to that obtained by maximal hormonal stimulation of the synthesis of ABP and transferrin by Sertoli cells. Levels of P Mod-S secreted into the medium by primary cultures of peritubular cells are increased in the presence of testosterone. Comparable concentrations of 17β-estradiol do not stimulate peritubular cells to synthesize P Mod-S. Data are interpreted to indicate that androgens act on testicular peritubular cells to increase the formation of P Mod-S and that P Mod-S may modulate the properties of adjacent Sertoli cells. Findings are discussed in relation to the nature of mesenchymal–epithelial cell interactions in the seminiferous tubule and to the possible role of P Mod-S as a mediator of androgen actions on Sertoli cells.**

Grobstein (1) was probably the first to put forward the concept that mesenchymal cells or their products are required for expression of the functions and morphology of adjacent epithelial cells during organogenesis. Since then, numerous investigations have demonstrated the validity of this concept (2, 3).

The viewpoint has been advanced "that the morphogenetic effects of hormones are linked to a requisite interaction of epithelium with stromal cells," and "that many hormone responses within epithelial cells may not be elicited directly in these target cells, but instead may be elicited by putative growth factors or inducers elaborated in neighboring stromal cells in response to hormonal stimulation" (3). Examples that support this general concept include the influences of mesenchymal cells on the morphogenesis of mammary epithelial cells (4, 5) and epithelial cytodifferentiation in the female and male genital tracts (3).

In the seminiferous tubules of adult testes, steady-state spermatogenesis is occurring, during which advanced germinal cells are continuously developing from stem cells (6). Restructuring of the seminiferous tubule cytoarchitecture takes place at discrete stages of the cycle of the seminiferous epithelium, correlated with the translocation of clones of germinal cells in early meiosis from the basal to the adluminal compartments (7). In this sense, the mature testis is undergoing developmental changes that resemble some of those occurring in other organs during fetal development (8).

The principal somatic cell types within the seminiferous tubule are the stromal (i.e., mesenchymal) peritubular cells (fibroblasts and myoid cells) and the epithelial Sertoli cells. Sertoli cells and peritubular cells interact with each other during coculture, leading to the formation of a basal lamina and a seminiferous tubule-like architecture (9). The presence of peritubular cells also results in an increased production of androgen-binding protein (ABP) by Sertoli cells (9, 10) and in an extended viability during coculture (9). To determine mechanisms by which peritubular cells might influence the properties of Sertoli cells, we have begun to search for components released by peritubular cells in culture that may alter the rates of synthesis of proteins produced by Sertoli cells (9, 11–14). In this communication, we report that protein(s) released by peritubular cells stimulate the production of ABP and transferrin by Sertoli cells and that the formation of the modulating protein(s) by peritubular cells appears to be regulated by androgens.

MATERIALS AND METHODS

Cell Culture and Medium Collection. Purified Sertoli cell-enriched aggregates were isolated from testes of 20-day-old Wistar rats by sequential enzymatic digestion, using procedures previously described (9, 11, 12). Decapsulated testis fragments (1.6 g) were digested for 25 min at 32°C in 50 ml of Hanks' balanced salt solution (HB) (GIBCO) containing trypsin (0.25%, GIBCO) and DNase (10 μg/ml, Sigma). Tryptic action was stopped by the addition of soybean trypsin inhibitor, after which the fragments were washed three times with HB. [Leydig cells are removed by trypsin digestion (11).] Tubule fragments were resuspended in 25 ml of HB containing collagenase (1 mg/ml, Sigma, Type I) and were incubated for 25 min at 32°C. Sertoli cell-enriched fragments were harvested by unit-gravity sedimentation, which leaves peritubular cells in the supernatant fraction (9, 11, 12). The fragments were washed with HB, and then digested for another 25 min in HB containing bovine testicular hyaluronidase (1 mg/ml, Sigma, Type I-S). Aggregates so obtained were washed three times with HB containing 0.1 mM EDTA, then twice in Ca^{2+}/Mg^{2+}-free HB with low-speed centrifugation between each wash, and then suspended in serum-free Eagle's minimal essential medium (ME medium) (GIBCO) supplemented as described (11). Cells were seeded in 24-well (1 ml per well) Linbro plates at $\approx 5 \times 10^5$ cells per well, corresponding to 1.3 μg of DNA/cm^2, and were maintained in culture in serum-free ME medium in the presence or absence of factors as indicated. The degree of contamination of these Sertoli cell-enriched preparations by peritubular cells was ≤0.3% (12).

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Abbreviations: ABP, androgen-binding protein; P Mod-S, proteins, secreted by peritubular cells, that modulate specific metabolic activities of Sertoli cells.

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Peritubular cells, isolated from the supernatant fraction of tubule fragments after digestion with collagenase (see above and ref. 12), were plated in 150-mm-diameter tissue culture plates containing 25 ml of 10% calf serum in ME medium and cultured for 5 days. Cells were removed by brief treatment with trypsin (1.7 mg/ml), washed, and replated at 1/4 density in 150-mm dishes. The subcultured cells grew to confluence in 4 days in ME medium containing 10% calf serum. Cells were then washed with serum-free medium, incubated for 6 hr in fresh serum-free medium, and washed again. Serum-free ME medium was added to these secondary cultures, and collections of conditioned medium were made at 48–72 hr intervals for periods up to 2 weeks. In other experiments, a mouse fibroblast cell line (3T3 cells) was grown to confluence in ME medium containing 10% calf serum, and then treated in the same manner as that described above for peritubular cells. Unless otherwise stated, peritubular-cell conditioned medium was that obtained from secondary cultures of cells isolated from testes of 20-day-old rats. In some experiments (Fig. 1*B*), secondary cultures of peritubular cells were prepared from testes of 10- or 60-day-old rats. In other experiments, conditioned medium was obtained from primary cultures of peritubular cells (see results described in Table 1 and Fig. 3). In all cases, conditioned medium collected was centrifuged (10,000 × *g* for 30 min at 4°C), and stored at −20°C.

To examine the effects of steroids on peritubular cells, primary cultures were seeded in the absence or presence of testosterone (10^{-6} M) or 17β-estradiol (10^{-6} M), and grown to confluence in 2 days in medium containing 10% calf serum. The medium was then removed and replaced with serum-free medium containing the steroid initially present, and collections of conditioned medium were begun. Various amounts of medium collected after the initial 72-hr period of peritubular cell culture in serum-free medium were added to Sertoli cells in culture. Influences of peritubular-cell-conditioned medium on Sertoli cells were assessed by determining the levels of transferrin produced by Sertoli cells, as described below.

Concentration and Fractionation of Peritubular-Cell-Conditioned Medium. The conditioned medium was concentrated and fractionated with an Amicon ultrafiltration system. Ultrafiltration membrane types YM-2, YM-10, XM-50, XM-100 and XM-300 with molecular weight exclusion limits of 2,000, 10,000, 50,000, 100,000, and 300,000, respectively, were used to concentrate the medium ≈200-fold. The concentrated samples were washed with 10 mM Tris Cl, pH 7.5, and centrifuged at 15,000 × *g* for 30 min at 4°C, and the supernatants were stored at −20°C.

Quantitative Assays of Products Synthesized by Sertoli Cells. Transferrin was analyzed by radioimmunoassay as previously described (13), utilizing a rabbit anti-rat transferrin antibody (Cappel Laboratories, Cochranville, PA) and a goat anti-rabbit immunoglobulin (Sigma) second antibody precipitation with polyethylene glycol. Plasminogen activator was analyzed with a plasminogen-dependent ^{125}I-labeled fibrin degradation assay (14). ABP levels were determined by radioimmunoassay, using the antibody and standard supplied by the National Hormone and Pituitary Program (National Institute of Child Health and Human Development, Bethesda, MD). Our ABP assay consisted of two incubations for 24 hr at 4°C. The first contained the sample, 30,000 cpm of ^{125}I-labeled ABP (15), rat ABP antibody (1:10,000 final dilution in 3 ml), and assay buffer [gelatin (2.5 mg/ml)/50 mM Tris Cl, pH 7.5/0.15 NaCl/1 mM EDTA] in a volume of 1.8 ml. Prior to the second incubation, 200 μl of goat anti-rabbit immunoglobulin (final dilution 1:600 in 3 ml) and 1 ml of polyethylene glycol buffer (polyethylene glycol 4000 at 132 mg/ml in 50 mM Tris Cl, pH 7.5) were added. After the incubations, the tubes were centrifuged at 2000 × *g* for 2 hr at 4°C, and radioactivity in the pellet was measured. The radioimmunoassay was linear in the range 1–30 ng of ABP, with a 10% coefficient of variation. The DNA content of culture wells was determined by the procedure of Karsten and Wollenberger (16) as previously described (17). All data for Sertoli cells were normalized per μg of cell DNA. Since the amount of DNA per confluent peritubular cell culture varied a maximum of 10%, the data were normalized per peritubular cell plate unless otherwise specified. Amounts of protein were determined with the Hartree procedure (18).

Additional Procedures. Proteolytic treatment of peritubular-cell-conditioned medium fractions was digestion with trypsin [5% (wt/wt)] for 6 hr at 37°C. The reaction was terminated by the addition of soybean trypsin inhibitor [10% (wt/wt)]. As a control, soybean trypsin inhibitor was added first to the trypsin solution, followed by addition of the protein sample in the same concentrations used above. Samples were incubated for 6 hr at 37°C and then assayed for stimulating activity on Sertoli cells in culture.

RESULTS

Stimulation by Conditioned Medium from Secondary Cultures of Peritubular Cells of Transferrin Production by Sertoli Cells. Levels of transferrin produced by Sertoli cell-enriched preparations in culture increased in proportion to the amounts added of conditioned medium from subcultured peritubular cells (Fig. 1*A*). Basal rates (40 ng of transferrin/μg of Sertoli cell DNA in 72 hr) were increased nearly 4-fold in the presence of 75% peritubular-cell conditioned medium (Fig. 1*A*). As a control, conditioned medium was ob-

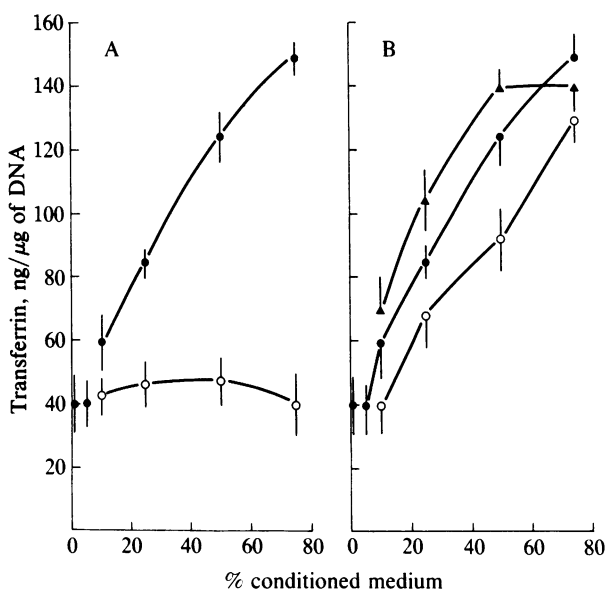

FIG. 1. Effects on transferrin production by Sertoli cells of various amounts of conditioned medium prepared either from secondary cultures of peritubular cells or from cultures of 3T3 cells. Purified Sertoli cell-enriched preparations were cultured in serum-free medium in the presence of indicated levels of serum-free peritubular cell- or 3T3 cell-conditioned medium, added at the time of plating, and replenished after 48 hr when the medium was changed. Levels of transferrin produced by Sertoli cells during the 72-hr period ending on day 5 were determined and expressed as ng of transferrin/μg of Sertoli cell DNA. Unless otherwise specified, Sertoli cell and peritubular cell preparations were from testes of 20-day-old rats. *(A)* Serum-free conditioned medium collected in the initial 72-hr period from secondary cultures of peritubular cells (●) or from 3T3 cells (○). *(B)* Conditioned medium for the 72-hr collection period terminating on day 5 from peritubular cells isolated from testes of 10- (○), 20- (●), or 60-day-old (▲) rats. Each point and error bar represents the mean ± SD (n = 9) for three separate experiments done in triplicate.

tained from confluent cultures of 3T3 cells during the first 72-hr collection period after removal of serum. No stimulatory factor was detected in components secreted by 3T3 cells (Fig. 1A). Samples of conditioned medium from secondary cultures of peritubular cells obtained from 72-hr collection periods on days 0–3 (Fig. 1A) and days 12–15 (not shown) of culture in serum-free medium both stimulated transferrin production by Sertoli cells. Conditioned medium from secondary cultures of peritubular cells prepared from testes of 10- or 60-day-old rats stimulated transferrin production (Fig. 1B) to the same extent as that observed with conditioned medium obtained from cells from testes of 20-day-old rats (Fig. 1 A and B).

Molecular Weight Fractionation of Peritubular-Cell-Conditioned Medium and the Influence of These Fractions on Sertoli Cells. Conditioned medium was obtained from secondary cultures of peritubular cells that had been maintained in serum-free medium for periods up to two weeks, with changes of medium every 48–72 hr. The pooled medium was concentrated by ultrafiltration, and the high molecular weight moieties were removed by filtration through an Amicon XM-300 (molecular weight exclusion limit 300,000) membrane. The filtrate was then sequentially fractionated through membranes with molecular weight exclusion limits of 100,000, 50,000, 10,000, and 2,000. Activities in each fraction were determined by measuring the effects on levels of transferrin, ABP, and plasminogen activator accumulated in the medium of cultures of Sertoli cells isolated from testes of 20-day-old rats. The concentrated pooled peritubular-cell-conditioned medium contained no detectable transferrin or ABP. The optimum time to measure the influence of peritubular cell factor(s) on transferrin and ABP production by Sertoli cells was found to be between days 2 and 8 of Sertoli cell culture (data not shown). In experiments reported here, the 72-hr medium collections between days 2 and 5 of Sertoli cell culture were analyzed.

Addition of the 50,000–100,000 molecular weight fraction of peritubular-cell-conditioned medium increased the levels of transferrin and ABP produced by Sertoli cells, whereas none of the other fractions elicited any stimulation (Fig. 2 A and B). None of the fractions from the peritubular-cell-conditioned medium increased levels of plasminogen activator, regardless of duration of treatment or time of collection (data not shown). Addition of a mixture of hormones (follicle-stimulating hormone, insulin, and testosterone) and retinol to the Sertoli cell cultures stimulated the formation of transferrin (Fig. 2A), ABP (Fig. 2B), and plasminogen activator (data not shown). These results confirm previous observations of the hormonal stimulation of the production of transferrin (13), ABP (19), and plasminogen activator (14) by conventional Sertoli cell-enriched preparations. The degree of enhancement of transferrin and ABP production elicited by the 50,000–100,000 molecular weight fraction of the peritubular-cell-conditioned medium was as great as that obtained with maximal stimulation by the mixture of hormones and retinol (Fig. 2 A and B). Combined data indicate that the 50,000–100,000 molecular weight fraction from peritubular-cell-conditioned medium contains component(s) that stimulate the production of transferrin and ABP by Sertoli cells but that do not increase the formation of plasminogen activator (Fig. 2 A and B).

Trypsin digestion of the 50,000–100,000 molecular weight fraction of the peritubular-cell-conditioned medium inactivated the component(s) that stimulate transferrin production by Sertoli cells (Fig. 2C). This loss of activity is most probably a consequence of proteolysis by trypsin, since pretreatment of trypsin with soybean trypsin inhibitor prevented inactivation (Fig. 2C). Heat treatment (95°C, 15 min) resulted in total loss of activity of peritubular-cell-conditioned medium (data not shown). To determine if the peritubular cell factor was either sensitive to or dissociated by acid, the conditioned medium was made 1 M in acetic acid upon collec-

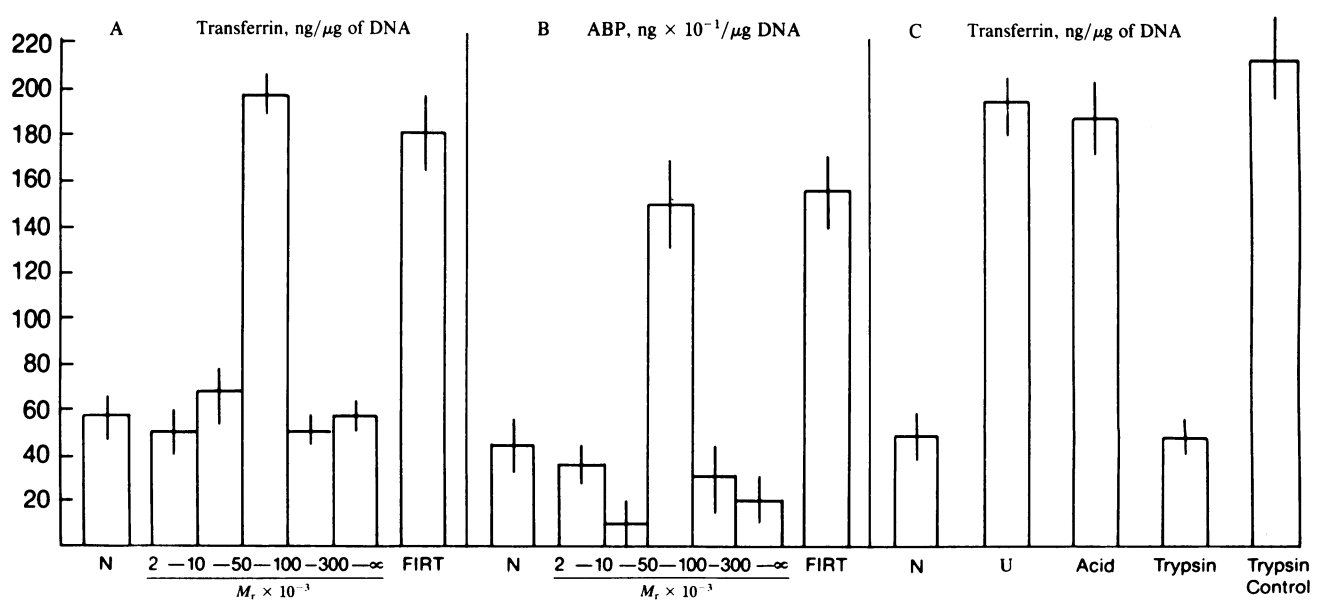

FIG. 2. Influence of fractions of conditioned medium from secondary cultures of peritubular cells on levels of transferrin and ABP synthesized by purified Sertoli cell-enriched preparations in culture. Sertoli cells were maintained in serum-free medium containing 25 μl of fractions (see text) indicated. Protein in 25-μl aliquots of the various molecular weight fractions: 2 μg, 2000–10,000; 4 μg, 10,000–50,000; 1.5 μg, 50,000–100,000; 1.7 μg, 100,000–300,000; and 4 μg, >300,000. Medium for the 72-hr period ending on day 5 of Sertoli cell culture was collected and analyzed for transferrin (A) or ABP (B). N, control cultures with no additions; FIRT, cells maintained in the presence of follicle-stimulating hormone (ovine NIH S-16, 100 ng/ml), insulin (5 μg/ml), retinol (0.35 μM), and testosterone (1 μM). (C) The influence of different treatments of the 50,000–100,000 molecular weight fraction on its stimulation of the synthesis of transferrin by Sertoli cells. Sertoli cells were untreated (N) or treated with the 50,000–100,000 fraction (1.5 μg of protein/25 μl) that had been untreated (U), obtained after initial acidification of peritubular-cell-conditioned medium (Acid), subjected to trypsin digestion for 6 hr prior to the addition of soybean trypsin inhibitor (Trypsin), or incubated for 6 hr with a mixture of soybean trypsin inhibitor and trypsin (Trypsin control). For details, see text. Each column represents the mean ± SD for three (C) or four (A and B) different preparations of peritubular cell fractions, each of which was analyzed in triplicate.

tion. After concentration and fractionation, the samples were lyophilized and reconstituted. The addition of the acid-treated fractions elicited responses by Sertoli cells identical with those observed after addition of fractions of the peritubular-cell-conditioned medium that had not been acid-treated (Fig. 2C). In both cases, only the 50,000–100,000 molecular weight fraction increased the production of transferrin by Sertoli cells. The retention of Sertoli-cell stimulating activity in samples of conditioned medium fractionated at a low pH eliminates consideration of a role for those protein-bound components, such as steroids, which would have become dissociated during the acid treatment.

The concentration of protein in the 50,000–100,000 molecular weight fraction peritubular-cell-conditioned medium required for half-maximal stimulation of transferrin production was 500 ng/ml under conditions described (Fig. 2A). In contrast, the concentration of protein in the 10,000–300,000 molecular weight fraction required for half-maximal stimulation was 4000–4500 ng/ml. These findings suggest a ≥8-fold purification of the active protein(s) in the conditioned medium by the sequential membrane filtration procedures. Subjection of these samples to gel filtration chromatography on Sephacryl S-200 results in a further increase in specific activity in the 70,000 molecular weight fraction (unpublished observations).

Stimulation by Testosterone of the Secretion of Active Components by Primary Cultures of Peritubular Cells. We compared the effects on transferrin production by Sertoli cells of various concentrations of conditioned medium from primary cultures of peritubular cells that had been maintained in basal ME medium in the presence or absence of testosterone (Fig. 3). Steroid-supplemented ME medium was used in the dilution of the steroid-treated peritubular-cell-conditioned medium, and all other Sertoli cultures were also treated with the appropriate steroid.

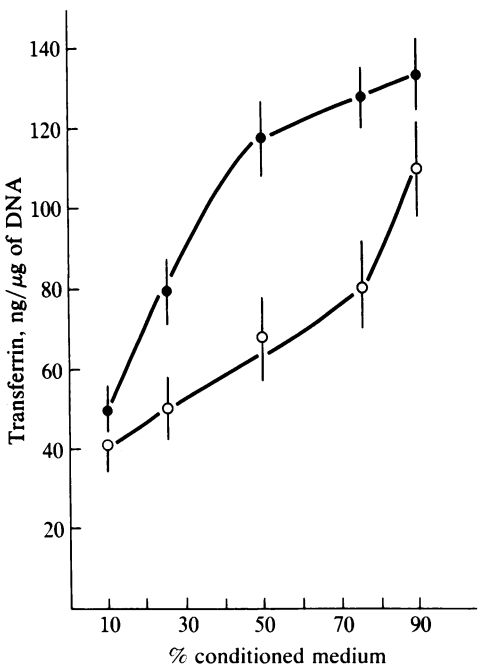

FIG. 3. Influence of androgens on secretion of active factor(s) by primary cultures of peritubular cells. Peritubular cells were cultured in the absence (○) or presence (●) of testosterone (10^{-6} M). Various concentrations of conditioned medium from the initial 72-hr collection of serum-free medium from primary cultures of peritubular cells were added to cultures of Sertoli cells. All Sertoli cell-enriched preparations were maintained in the presence of 10^{-6} M testosterone. Medium from the 72-hr collection on day 5 of the Sertoli cell culture was analyzed for transferrin. Points and error bars represent mean ± SD (n = 9). Three separate experiments were performed on control and testosterone-treated cultures, each of which was analyzed in triplicate.

Control preparations of purified Sertoli cell-enriched aggregates produced the same amounts of transferrin when cultured in the presence or absence of steroids (40 ± 6 ng/μg of cell DNA in 72 hr). These data confirm previous observations, which show minimal effects of testosterone alone on transferrin synthesis (13). Addition to the Sertoli cell medium of an equal volume of conditioned medium from primary cultures of either untreated or 17β-estradiol-treated peritubular cells increased transferrin production to the same extent (Table 1). Note that the degree of Sertoli cell stimulation elicited by conditioned medium from control primary cultures (Fig. 3) was less than that observed after addition of comparable quantities of conditioned medium from secondary cultures of peritubular cells (Fig. 1). Conditioned medium from primary cultures of peritubular cells maintained in the presence of testosterone was more potent in stimulating Sertoli cells to produce transferrin (about a 150% increase when the concentration of conditioned medium was 50%) (Table 1 and Fig. 3). Since the same amount of testosterone was present in all Sertoli cell cultures in experiments performed in this series, a possible synergism of testosterone and the peritubular cell factor(s) on transferrin production can be excluded.

Stimulation by androgens of peritubular cells in primary culture was consistently obtained when the protocols described were rigorously adhered to. However, inconsistent effects of androgens were obtained in primary cultures of peritubular cells maintained for >5 days after removal of serum from the medium and in secondary cultures of peritubular cells. Amounts of stimulatory activity present in conditioned medium from testosterone-treated short-term primary cultures were similar to those detected in control long-term primary or secondary cultures (Figs. 1 and 3). This implies that, as basal rates of production of the peritubular cell modulating protein(s) increase during time in culture, the stimulating effects of testosterone diminish.

DISCUSSION

Our results show that testicular peritubular cells in culture release components into the medium that modulate functional activities of purified Sertoli cell-enriched preparations. Preliminary fractionation has indicated that the active factor(s) is an acid-stable, trypsin-sensitive, heat-sensitive protein(s) having an apparent molecular weight between 50,000 and 100,000 (Fig. 2). We shall refer to the active factor(s) as P Mod-S, to indicate protein(s), produced by peritubular

Table 1. Stimulation by testosterone but not 17β-estradiol of secretion of active component(s) by primary peritubular cells

% conditioned medium	Transferrin, ng/μg of Sertoli cell DNA		
	Control	Testosterone, 10^{-6} M	17β-estradiol, 10^{-6} M
0	40 ± 6	48 ± 7	41 ± 6
50	68 ± 9	120 ± 13	69 ± 12

Primary cultures of peritubular cells were maintained in the absence or presence of testosterone or 17β-estradiol. Serum-free conditioned medium from the initial 72-hr collection period from primary peritubular cell cultures was added to half of the purified Sertoli cell-enriched preparations at the time of their plating; the other Sertoli cell preparations were maintained in the absence of peritubular cell conditioned medium. Sertoli cells in the presence or absence of conditioned medium were cultured in the presence of steroids indicated. The 72-hr collection period medium obtained on day 5 of Sertoli cell culture was analyzed for transferrin. Data, expressed as mean ± SD (n = 9), represent results from three separate experiments, each analyzed in triplicate. For details, see text.

cells (P), that modulate specific metabolic activities of Sertoli cells (S). Addition of P Mod-S alone stimulated Sertoli cells to synthesize ABP and transferrin to an extent as great as that observed in cells maximally stimulated by a mixture of follicle-stimulating hormone, insulin, retinol, and testosterone. The production of transferrin by Sertoli cell-enriched preparations in culture is enhanced to various degrees by follicle-stimulating hormone, insulin, retinol, or testosterone, but maximal stimulation requires the combined effects of all four (13). Transferrin production by Sertoli cells was increased to a greater extent by P Mod-S than by any of the four alone.

P Mod-S is most probably not identical to any of the known growth factors of the platelet-derived growth factor, epidermal growth factor, or insulin families, since all of these have molecular weights <31,000 (for review, see ref. 20), and none would have appeared with P Mod-S in the 50,000–100,000 molecular weight fraction after acidification (Fig. 2). P Mod-S activity cannot be attributed to luteinizing hormone or human chorionic gonadotropin, since neither influences any of several Sertoli cell functions previously examined (11, 21).

Peritubular cell structure has been described in detail, but their functions are less well defined (22). They serve as a nonspecific barrier in the boundary tissue of the seminiferous tubule (22). Peritubular cells have been implicated in the contractions of seminiferous tubules (23). Androgen receptors are present on these cells (24, 25), and our observations (Table 1 and Fig. 3) indicate that peritubular cells respond to androgens. We postulate that P Mod-S production is involved in mediating androgen effects on Sertoli cell functions. Additional evidence in support of this possibility has recently been obtained in experiments on purified populations of Sertoli cell-enriched aggregates to which various numbers of peritubular cells have been added. Androgen stimulation of ABP production by Sertoli cells is enhanced by the presence of peritubular cells (unpublished results).

Peritubular cells act cooperatively with Sertoli cells in coculture to form a basal lamina, elicit the formation of a seminiferous tubule-like morphology, and prolong survival (9). Peritubular cells cultured alone synthesize fibronectin, whereas Sertoli cells do not (12). Our results show that P Mod-S produced by peritubular cells under androgenic control stimulates the synthesis of transferrin and ABP by Sertoli cells. We conclude that peritubular cells have the capacity to modulate several Sertoli cell functions. We speculate that these two somatic cells of the seminiferous tubule may form a functional unit that plays an integral role in the maintenance of the cytoarchitectural arrangements and the biochemical environment in the seminiferous tubule required for spermatogenesis to proceed (21).

The general hypothesis has been developed that mesenchymal cells under steroid control can synthesize proteins that influence the function and morphogenesis of epithelial cells (3). The present study provides direct evidence that mesenchymal cells (seminiferous peritubular cells) under steroid control (testosterone) synthesize a protein(s) (P Mod-S) that can modify the functions of neighboring epithelial cells (Sertoli cells). Isolation and characterization of P Mod-S, together with continuing investigations of peritubular cell–Sertoli cell interactions, may increase understanding of the role of mesenchymal–epithelial cell interactions in the regulation of testicular functions.

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1. Grobstein, C. (1967) *Natl. Cancer Inst., Monogr.* **26,** 279–299.
2. Hay, E. D. (1981) in *Cell Biology of Extracellular Matrix*, ed. Hay, E. D. (Plenum, New York), pp. 379–409.
3. Cunha, G. R., Chung, L. W. K., Shannon, J. M., Taguchi, O. & Fujii, H. (1983) *Recent Prog. Horm. Res.* **39,** 559–595.
4. Kratochwil, K. (1969) *Dev. Biol.* **20,** 46–71.
5. Durnberger, H. & Kratochwil, K. (1980) *Cell* **19,** 465–471.
6. Clermont, Y. (1972) *Physiol. Rev.* **52,** 198–236.
7. Russell, L. (1977) *Am. J. Anat.* **148,** 313–328.
8. Roosen-Runge, E. C. (1977) in *The Process of Spermatogenesis in Animals* (Cambridge Univ. Press, Cambridge, U.K.), pp. 1–12.
9. Tung, P. S. & Fritz, I. B. (1980) *Biol. Reprod.* **23,** 207–217.
10. Hutson, J. C. & Stocco, D. M. (1981) *Endocrinology* **108,** 1326–1368.
11. Dorrington, J. H., Roller, N. F. & Fritz, I. B. (1975) *Mol. Cell. Endocrinol.* **3,** 57–70.
12. Tung, P. S., Skinner, M. K. & Fritz, I. B. (1984) *Biol. Reprod.* **30,** 199–211.
13. Skinner, M. K. & Griswold, M. D. (1982) *Biol. Reprod.* **27,** 211–221.
14. Lacroix, M., Smith, F. E. & Fritz, I. B. (1977) *Mol. Cell. Endocrinol.* **9,** 227–236.
15. Gunsalus, G. L., Musto, N. A. & Bardin, C. W. (1978) *Science* **200,** 65–66.
16. Karsten, U. & Wollenberger, A. (1977) *Anal. Biochem.* **77,** 464–470.
17. Louis, B. G. & Fritz, I. B. (1979) *Endocrinology* **104,** 454–461.
18. Hartree, E. F. (1972) *Anal. Biochem.* **48,** 422–427.
19. Fritz, I. B., Rommerts, F. F. G., Louis, B. G. & Dorrington, J. H. (1976) *J. Reprod. Fertil.* **46,** 17–24.
20. Heldin, C.-H. & Westermark, B. (1984) *Cell* **37,** 9–20.
21. Fritz, I. B. (1978) in *Biochemical Actions of Hormones*, ed., Litwack, G. (Academic, New York), Vol. 5, pp. 249–281.
22. Dym, M. & Fawcett, D. W. (1970) *Biol. Reprod.* **3,** 308–326.
23. Hovatta, O. (1972) *Z. Zellforsch.* **131,** 229–308.
24. Sar, M., Stumpf, W. E., Mclean, W. S., Smith, A. A., Hansson, V., Nayfeh, S. N. & French, F. S. (1975) *Curr. Top. Mol. Endocrinol.* **2,** 311–319.
25. Verhoeven, G. (1980) *J. Steroid Biochem.* **13,** 469–474.